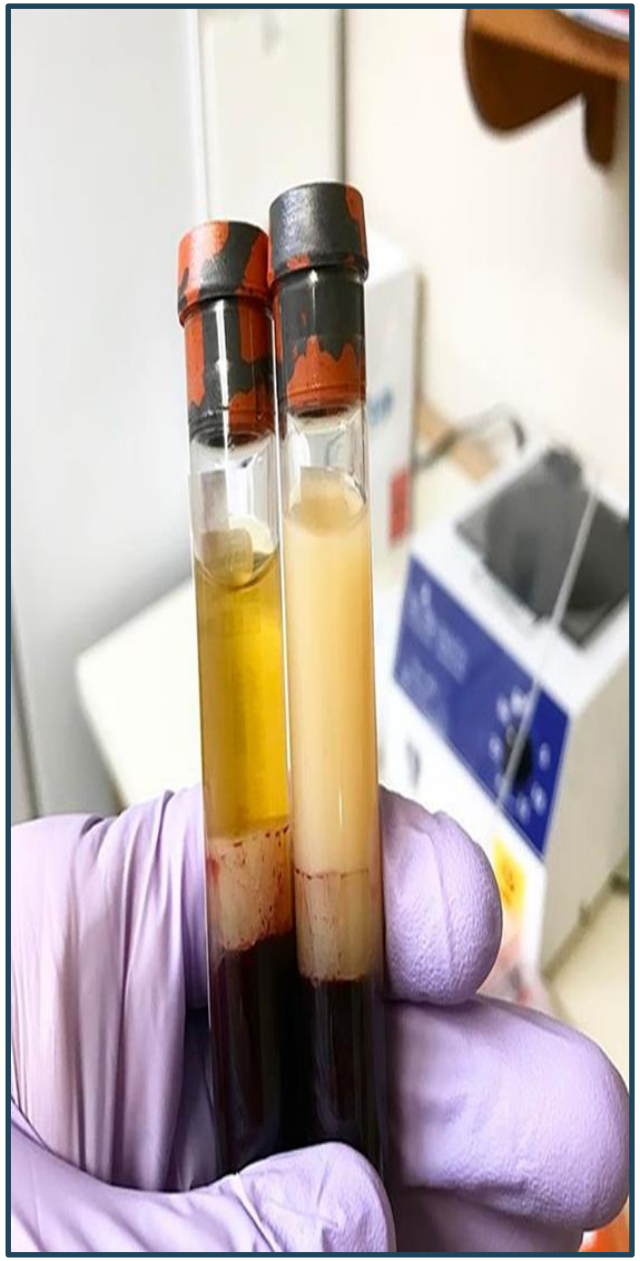
Institute of Chemistry
Faculty of natural sciences and mathematics
Ss "Kiril i Metodij University"-Skopje, Macedonia

Voltammetric sensor for direct quantification of bilirubin, uric acid and albumins in human serum



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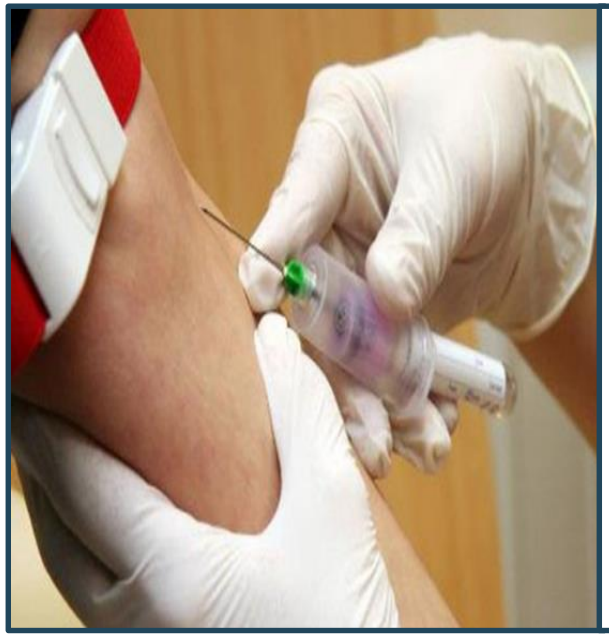
Student: Msci Pavlinka Kokoskarova

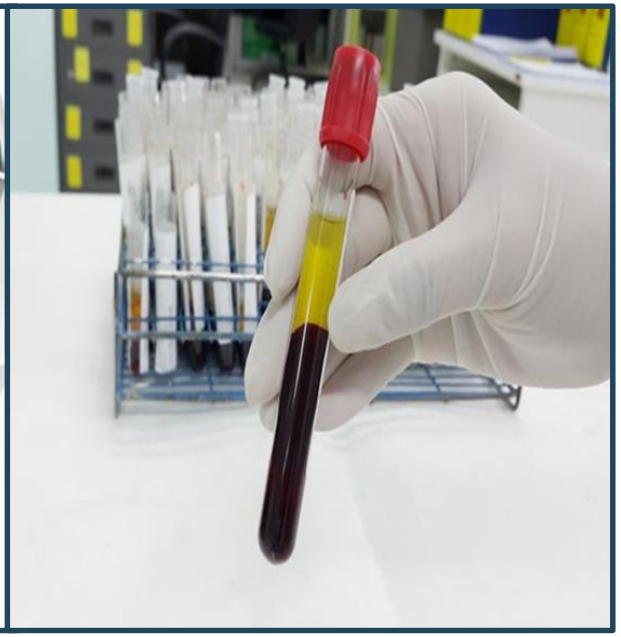
Human Serum



venipuncture

Vacutainer tubes with coagulation activator

centrifugation



Blood serum

SERUM-is a blood system that contains proteins, antigens, antibodies, hormones, electrolytes and other substances that do not contribute to the blood cloothing.

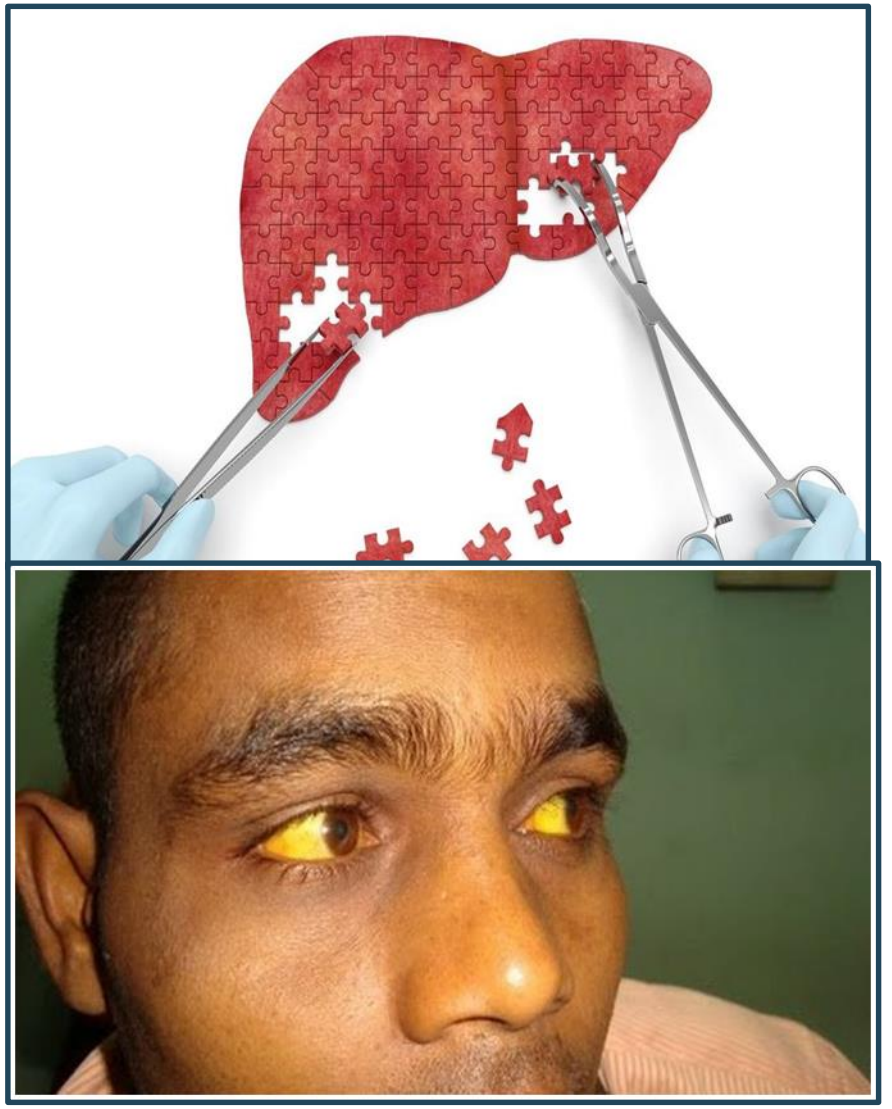
Bilirubin

- Is a degradation product of hem-containing proteins;

- 80 %-originates from hemoglobin
- 20%-comes from myoglobin, catalase enzymes, and peroxidase enzyme;

- Diazo reaction (DSA) is a specific reaction for Detection of bilirubin.

TOTAL bilirubin= direct + indirect



icterus

Uric Acid

(acidum uricum)

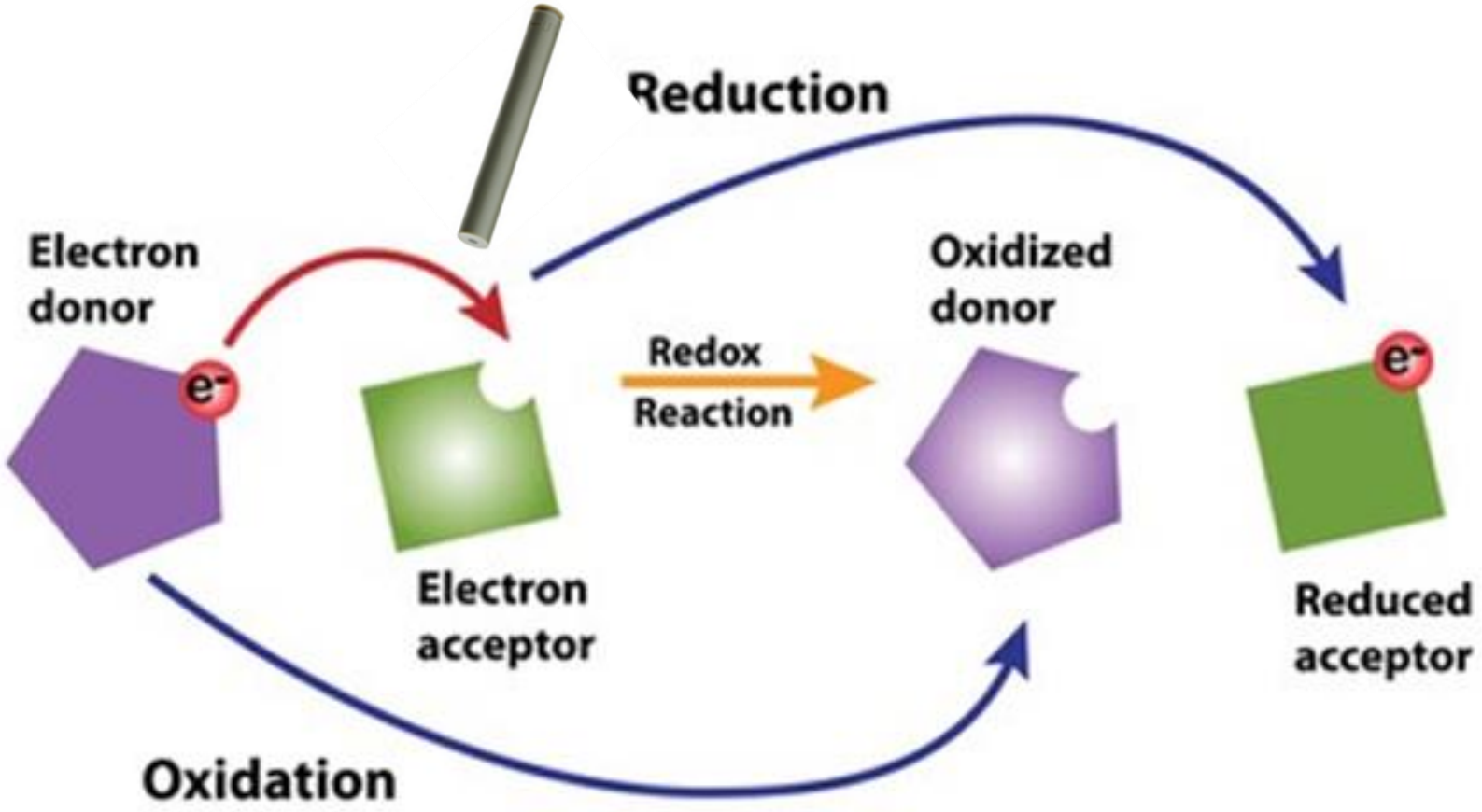
- Final product of purine nucleosides degradation;
- pH 5,75 ;
- Under physiological pH – content of sodium urate up to 416 µmol/L;
- Under conditions when uric acid content is higher than 416 µmol/L-giht (urolitiase) is the name of the disease;

Albumins

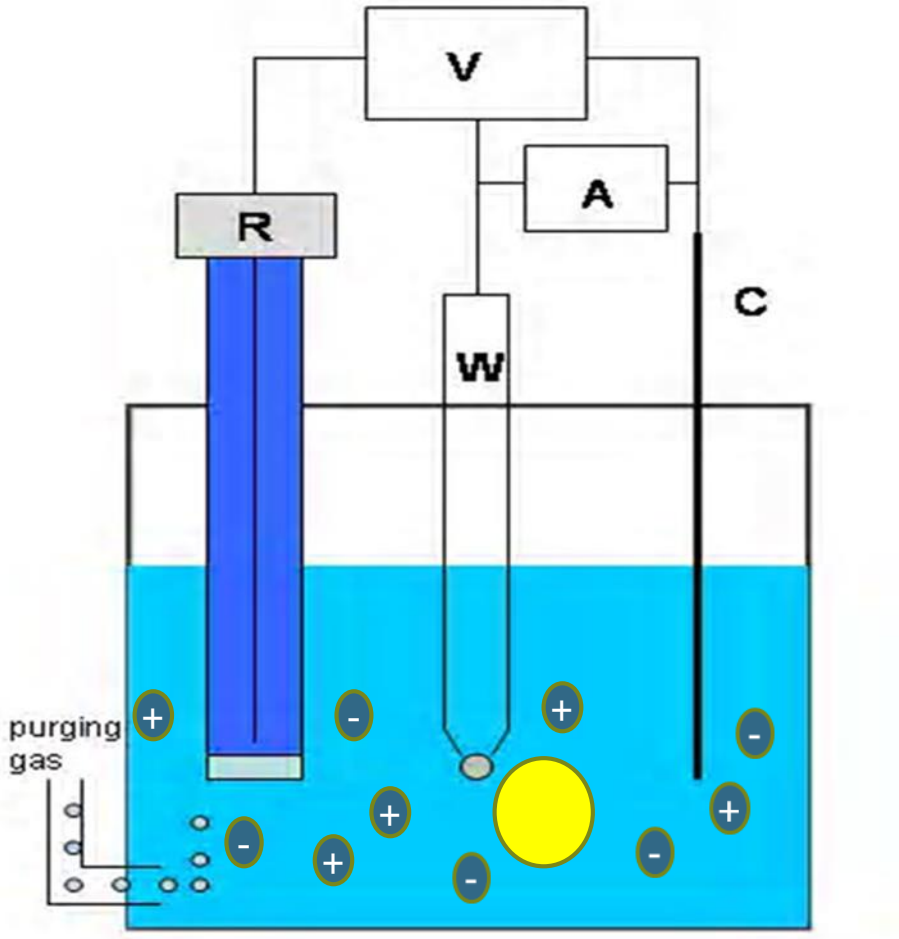
- Blood plasma proteins with lowest molecular weight;
- Their synthesis occurs in the liver;
- Albumins take part in transport of metabolites, hormones, drugs…
- They are important systems to maintain osmotic pressure and acid-base equilibria in the cells;
- Specific detection of albumins can be achieved via reaction with bromcresol green (BCG).

Voltammetric techniques

– are electrochemical techniques suitable to study the processes of oxidation and reduction of given analyte, where so-called "working electrode" is one partner in the electron exchange with the studied analyte.



Electrochemical cell

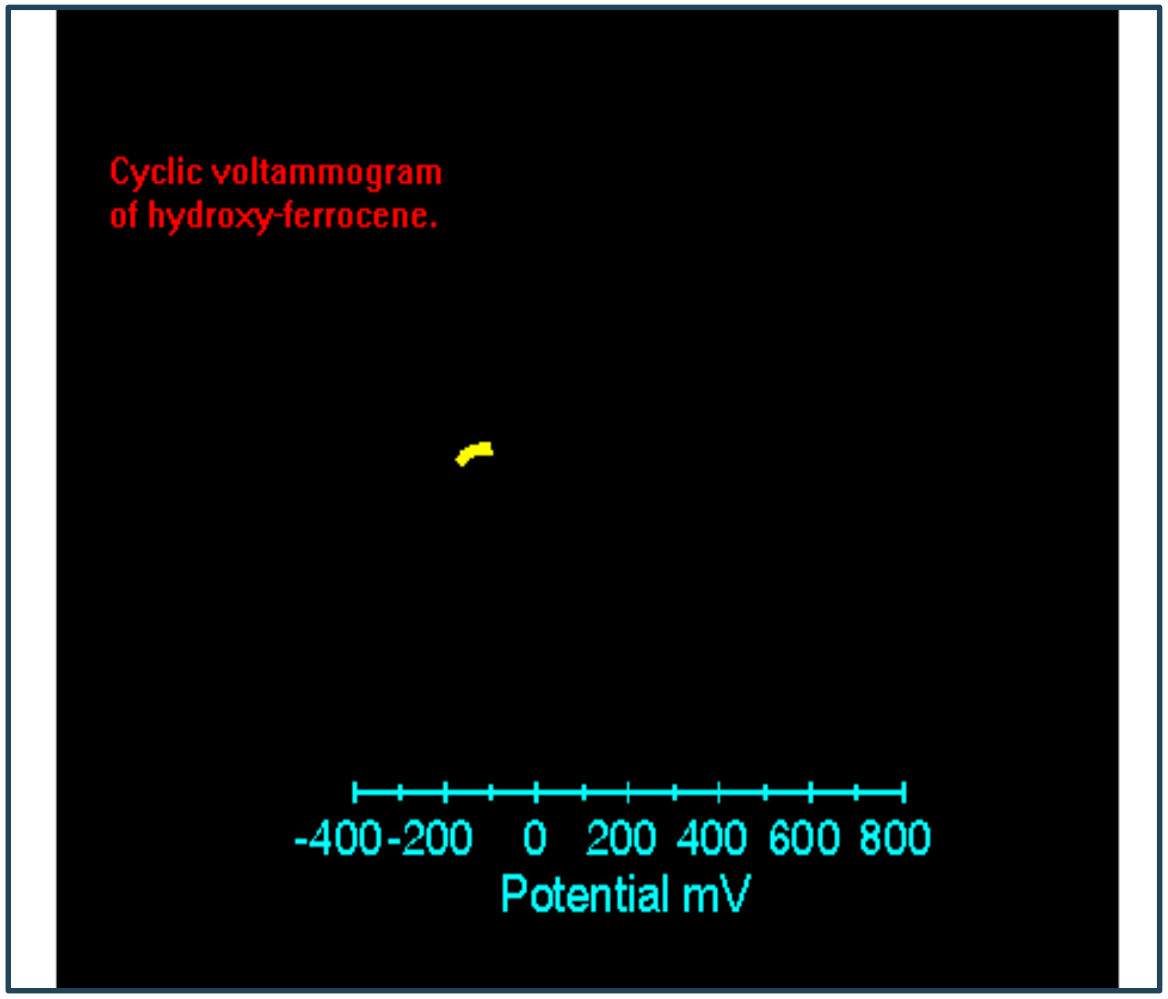


- -a glass container that contains an analyte whose molecules can be reduced or oxidized at the working electrode;

- E;ectrp;yte in which three electrodes are submerged
  - -working electrode (W)
  - -reference electrode (R)
  - -counter electrode (C);

- From outside source one applies a **potential difference between the working and the reference electrode** (this is a driving force for the electrons from the outer shells of the working electrode) and electric current is measured that is a result of the electron exchange between the working electrode and the studied analyte.

Cyclic Voltammetry

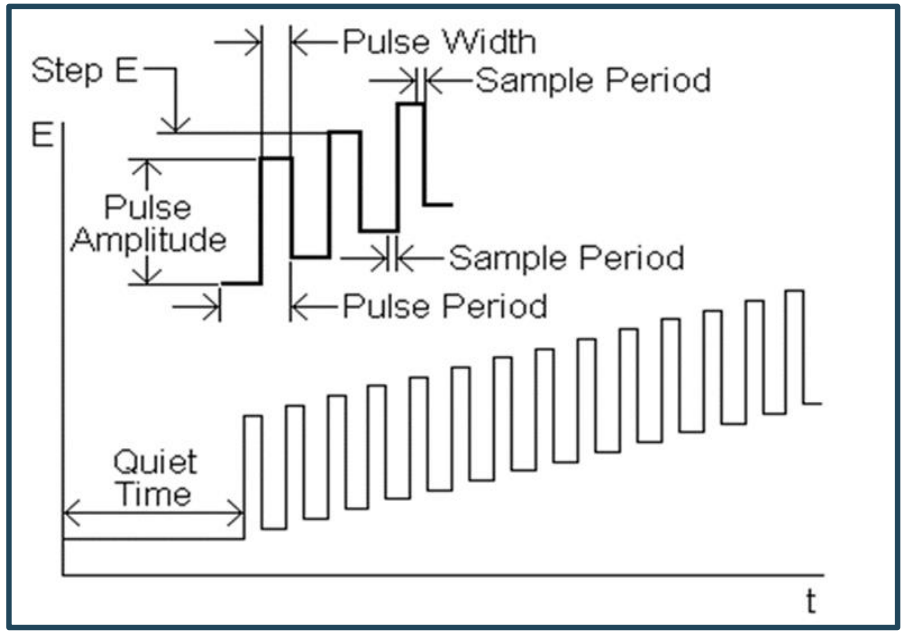


- The most common voltametric technique
- By changing the potential difference from starting negative to final positive potentials one can follow processes of **oxidation**;

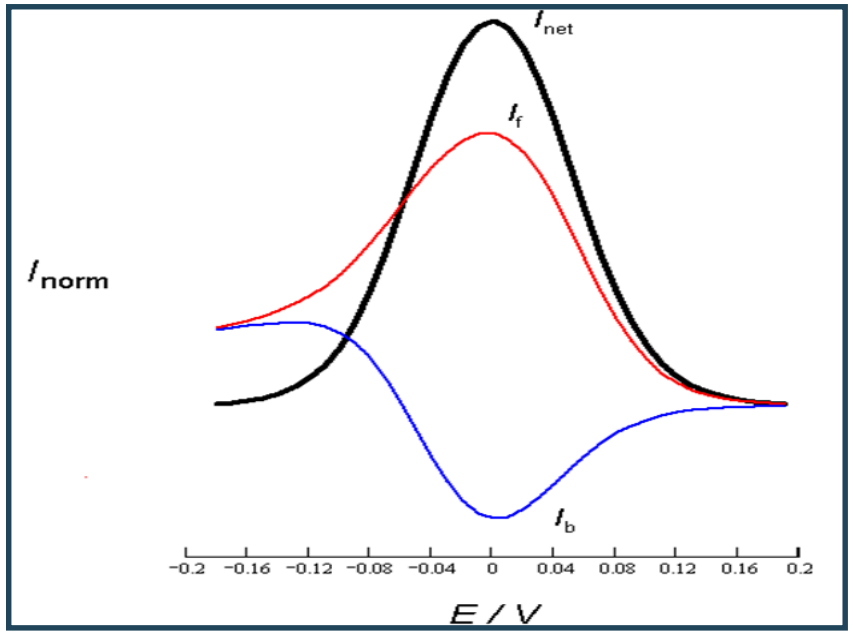
- In opposite scenario, by running the potential from positive to final negative potentials one follows processes of **reduction**;

- **Cyclic voltammogram is final output of a cyclo voltammetric experiment**.

Pulse Voltammetric Techniques



- Potential is applied in a form of defined pulses;
- Current is measured at the end of potential pulses;
- This measuring manner minimazies the undesired capacitance current, while mainly Faradaic current are detected.



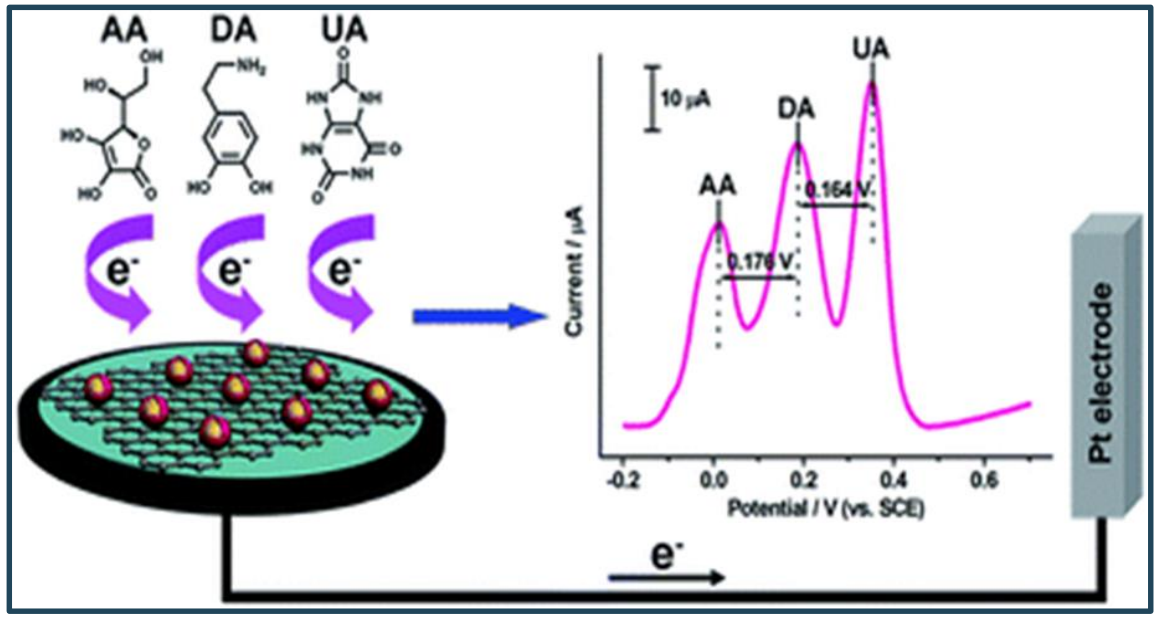
- Square-wave voltammetry (**SWV**) is the most advanced among pulse voltammetric technques;
- It provides insight into both half-reaction of oxidation and reduction, and it contains a NET current which is defined as a sum of absolute values of the oxidation and reduction currents

Voltammetric biosensor

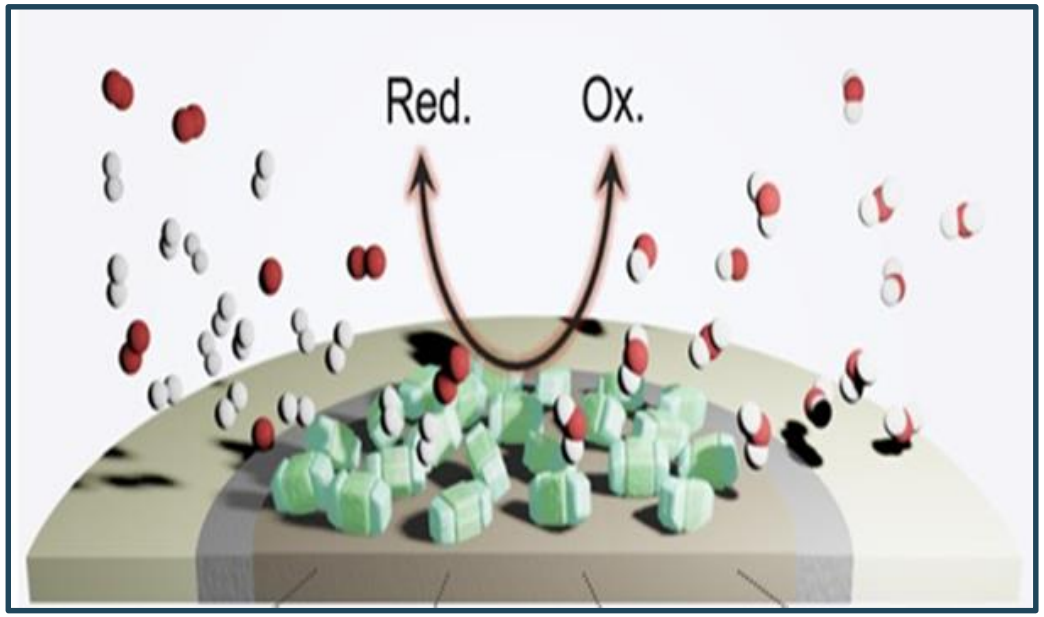
- Is is an amperometric electrochemical system that should measure the Faradaic current as a linear function of the analyte concentration .

- Major criteria in sensor design are:

-sensitivity;
-specificity;
-linearity;
-velocity of obtaining an instrumental answer;
-reproducibility.



Scheme of an amperometric biosensor



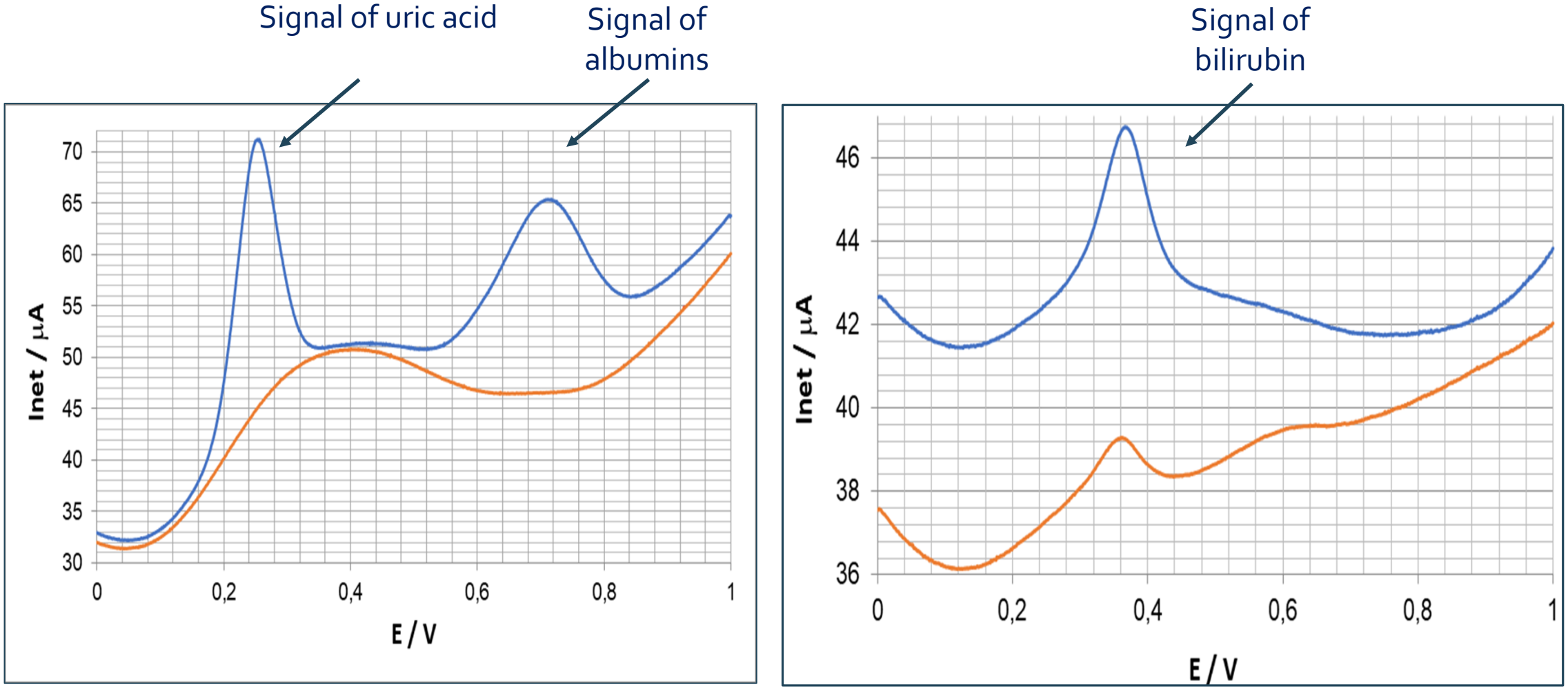
Modification of electrode surface with nano-materials...often practiced to achieve a better sensitivity

Some literature data

In the literature, there are more than 900 papers published for single detection of uric acid, bilirubin and albumins in human serum, or in combination with vitamin C, hemoglobin, glucose, dopamine, and amino acids cysteine and tyrosine.

Major techniques used	Working electrode	Modifiers of working electrode surface	Selectivity of the sensors is achieved via…	Our task
-cyclic voltammetry; -adsorptive stripping differential pulse voltammetry.	Glassy Carbon Electrode (GCE).	-carbon nanoparticles; -metallic nanoparticles.	-application of defined enzyme at the working electrode surface, which is specific for given substrate only	-to achieve a simultaneous determination of uric acid, bilirubin and albumins by using square-wave voltammetry.

Preliminary results with square-wave voltammetry



Direct voltammetry of human serum with SWV performed by using edge place pyrolytic graphite electrode (EPPGE) as working electrode

Major focus on the investigations in the doctoral thesis will be :

- Optimization of experimental conditions;
- Study of the effect of different nanomaterial;
- Study of possible interferences;
- Correlation of obtained results with the pathology of patients;
- Design of electronic system for practical application of the sensor
- Statistical analysis
- …

Expected outputs from the voltammetric sensor designed for direct simultaneous determination of uric acid, bilirubin and albumins

- Fast instrumental response in micromolar concentrations of analytes
- Use of simple and cheap experimental system (instrumentation)
- Possibility of fast simultaneous determination of the analytes from biological samples

THANK YOU
FOR YOUR
ATTENTION

LITERATURE:

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12. Gulaboski, Rubin and Markovski, Velo and Zhu, Jihe, ***Journal of Solid State Electrochemistry,*** 20. pp. 1-10. *ISSN 1432-8488 Redox chemistry of coenzyme Q—a short overview of the voltammetric features.* 20 (2016) 3229-3238

13. Rubin Gulaboski, Valentin Mirceski, New aspects of the electrochemical-catalytic (EC') mechanism in square-wave voltammetry, ***Electrochimica Acta***, *167, 2015, 219-225.*

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